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Biological Effect of Tiger Nut (*Cyperus esculentus L.*) Oil on Healthy and Hypercholesterolemia Rats

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Abstract

The present work aimed to study the effect of tiger nut oil on healthy and hypercholesterolemia rats, including feeding, growth parameters and biological analysis. Forty-eight male albino rats weighting 150 ±5 g were divided into eight homogenous groups, four groups (T_1, T_3, T_5 and T_7) were healthy. One of these groups was chosen as a negative control group (T_1). The rats in negative control group fed on basal while the three remaining groups of rats, fed on basal diet with different levels of tiger nut oil (5, 10 and 15%) for 4 weeks. While the other four groups (T2, T_4, T_6 and $T_{8)}$ considered as hypercholesterolemia. One of these groups was chosen as a positive control group (T_2), where T_2 group fed on basal diet enriched with 1% cholesterol and 0.5% colic acid. The three remaining groups of rats fed on basal diet enriched cholesterol with different levels of tiger nut oil (5, 10 and 15%) for 4 weeks. The results revealed that all hypercholesterolemia groups which feed on 5%, 10%, and 15% of tiger nut oil resulted a varied increase in body weight gain, good intake and growth rate. Results declared that there was a significant difference ($P \leq 0.05$) between the positive control group and cholesterol emic group treated with 10% and 15% tiger nut oil in internal organ weights. Whereas, data showed that there was no significant difference ($P \geq 0.05$) between the negative control group, and healthy rat groups. The results declared a significant decrease in GOT, GPT enzymes activity, creatinine, blood urea and uric acid for treated groups as compared with healthy rat groups or hypercholesterolemia rats group. Results indicated that hypercholesterolemia rat groups, which treated with 10 or 15% tiger nut oil resulted in a significant decrease ($P \leq 0.05$) in the values of serum total lipids, total cholesterol, T.G, LDL-cholesterol, vLDL. LDL-cholesterol and atherogenic index (AI), but showed a significant increase ($P \leq 0.05$) in the values of serum HDL-cholesterol. Fatty acid composition of tiger nut oil made it ideally suited as a nutritional defense against lipid oxidation. Hence, the study recommended using tiger nut oil meal-based diets to overcome the problem of hypercholesterolemia beside improving the liver and kidney functions.

Key words: Biological properties, Chemical composition, *Cyperus esulentus* oil, Hypercholesterolemia.

Introduction:

Hyperlipidemia is a disease resulting from the high fat diets. It is a powerful and extremely one of

the major causes of the development of cardiovascular disorders (Jain *et al*., 2010). In Egypt, it is an important public health problem, as well as in many parts of the world. Hyperlipidemia characterized by elevated serum total cholesterol, low density, very low-density lipoprotein and decrease high density lipoprotein, which are the risk factor for coronary heart disease (Luqman *et al*., 2012).

Hyperlipidemia was classified into a primary and a secondary type, which indicates the complexities associated with the disease. The primary disease may be treated using antilipidemic drugs, but the secondary type originating from renal lipid nephrosis or hypothyroidism, which demands the treatment of the original disease rather than hyperlipidemia (Anthony and Ashawe, 2014). Hyperlipidemia is leading preventable cause of death worldwide, with increasing prevalence in adults and children, and authorities view and it as one of the most serious public health problems of the 21 centuries (El-Bushuty and Shanshan, 2012). The treatment of hyperlipidemia depends on the patient's cholesterol profile. Many antihyperlipidemic agents like stating, niacin, bile acids, ezitimibe etc., reduce cholesterol level in different condition (Mohamed, 2016).

Nowadays, there is an increased consumption of *C. esculentus* and *Moringa oleifera* oils all over the world, since many peoples have realized the health risks that result from increased consumption of high fat diets. Tiger nut belongs to the Division: Mangoliophyta; Class: Liliopsida; Order: Cyperales; and Family: Cyperaceae (Muhammad *et al*., 2011). Tiger nut is commonly known as earth almond, chufa, yellow nut sedge and Zulu nuts. Chufa produces rhizomes from the base with somewhat spherical tuber. In Egypt, it is used as a source of food, medicine and perfumes (Imam *et al*., 2013) .*Cyprus esculentus* oil was reported to help in preventing heart attacks, thrombosis and activates blood circulation (Bamishaiye *et al.,* 2010), responsible for preventing and treating urinary tract, assist in reducing the risk of colon cancer (Gambo and Da'u, 2014), for preventing or reducing bad cholesterol (LDL-cholesterol) and increasing the good cholesterol (HDL-cholesterol) transporting the cholesterol placed in the arteries to the liver for its destruction, reducing triglyceride level in blood, because of its content in oleic acid and polyunsaturated fatty acid (Mohamed, 2016). The oil of tiger nut is used to reduce cholesterol or lose weight (Imam *et al.,* 2013), and beneficial to diabetics mellitus (Adejuyitan, 2011), since its consumption can help to prevent heart problems and activate blood circulation. Tiger nut oil is highly unsaturated fatty acids and good for the health of humans. Tiger nut tuber was reported as healthy and it helps in preventing cancer, due to high content of soluble glucose (Muhammad *et al*., 2011; Samuel *et al.,* 2102; Imam *et al*., 2013). In the current study we attempt to carry out comprehensive hypocholesterolemic profiling of tiger nut oil, which are grown in many parts of Egypt. Herein, the present study was designed to evaluate the effect of feeding different levels of tiger nut oil (5, 10 and 15%) on healthy and hypercholesterolemic rat groups on serum lipid profiles.

Materials and Methods:

Tiger nuts were purchased from an open market in Assiut, Egypt. Forty-eight adult male albino rats, weighting (150 ± 5g) were obtained from the animal house, Faculty of Medicine, Assiut University. All chemicals and reagents used were of analytical grade from Merck.

Tiger nut oil was extracted from the milled tiger nut using Soxhlet extractor as described by AOAC (2005).

Animal experimental design:

The rats had been fed on commercial non-purified diet, after adaptation period for four days to the new environmental conditions. The rats were divided into eight groups (n=6). The animals were housed individually in plastic metabolic cages in a temperature and humidity controlled room (25° C with a 12 hrs light-dark cycle).

Experimental diets:

The rats were fed on basal diet according to Khalil, (2004) and classified as follows:

T_1: Rats fed on basal diet (Negative control), supplemented with corn oil 10%.

T_2: Rats fed on basal diet (Positive control), supplemented with corn oil 10%, beside 1% cholesterol and 0.5% colic acid.

T_3: Rats fed on basal diet supplemented with 5% tiger nut oil.

T_4: Rats fed on positive diet beside 5% tiger nut oil.

T_5: Rats fed on basal diet supplemented with 10% tiger nut oil.

T_6: Rats fed on positive diet beside 10% tiger nut oil.

T_7: Rats fed on basal diet supplemented with 15% tiger nut oil.

T_8: Rats fed on positive diet beside 15% tiger nut oil.

The basal diet consists of protein (10%), corn oil (10%), choline chloride (0.2%), cellulose (5%), vitamin mixture (1%), salt mixture (4%) and starch (up to 100%). (El-Bushuty and Shanshan, 2012). Food and tap water provided ad libitum for 28 days. Body weight gain and food intake were recorded periodically. Blood samples were collected in fasting conditions by killed under mild anesthesia and the organs were collected (liver, heart and kidney). The blood samples were collected at zero time and after 28 days and allowed to clot and the serum was stored in deep-freeze for further analysis.

Biochemical analysis:

Glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) enzyme activities in rat serum were measured according to Reitman and Frankel, (1957). Blood urea and uric acid were measured as described by Patton and Crouch, (1977). Creatinine content in rat serum was determined according to the method described by Owen *et al.,* (1954); total cholesterol by Lopes-Virella *et al*., (1977); LDL-cholesterol was calculated according to the method described by Friedewald *et al*., (1972) as follow:

vLDL-cholesterol (mg/dl) =

LDL-cholestrol (mg/dl) =

Total cholesterol – (vLDL + HDL-cholesterol)

Triglyceride level was estimated according to the method described by Uwajima *et al.,* (1984). Serum total lipids were determined according to the method described by Frings and Dunn, (1970).

Tocopherol analysis:

Tocopherols content (α, β, γ and δ) in corn and tiger nut oil samples were determined by high-performance liquid chromatography (HPLC). Samples were treated according to the method described by Ueda and Igrashi, (1990) and Neff *et al.,* (2003). The tocopherol peaks were identified by predetermining the retention times of individual tocopherol standards and results. A linear standard curve of areas for tocopherol standards from concentration of 0.6 – 500 ppm was obtained to calculate ppm., then expressed as mg/100g oil.

Hydrocarbon and sterol fractions:

The un-saponifiable matter was extracted from oils after saponification at ambient temperature according to the method reported by Abd-El-Ghany**,** (2006). The un-saponifiable matter was analyzed for hydrocarbons and sterol substances using GC-MS apparatus with a flame ionization detector. The column used for separating the un-saponifiable matter was (30m × 0.53mm). (I.D.) 0.88 µm film thickness fused silica capillary column HPI (methyl silicone).

Fatty acids composition:

The oil samples were prepared to yield fatty acid methyl esters (FAMEs). Fatty acids composition were determined by GC-MS equipped with flame ionization detector. The peak was measured by

triangulation and the relative proportion of individual compound obtained by determination the partial areas in relation to total area. **(**Samuel *et al.,* 2012).

Statistical analysis:

The data were subjected to statistical analysis using the method of student "t" test (User Guide Statistical Analysis System SAS, 2000). Significant differences were determined at 0.05 level of Probability.

Results and Discussion:

Un-sapnifiable matters and tocopherol compounds of tiger nut and corn oils:

Data presented in Table (1) shows the tocopherol compounds of tiger nut and corn oils. Un-saponifiable matters content of tiger nut oil was higher (0.70%) than that of corn oil (0.5%). Un-saponifiable matter (includes hydrocarbons, sterols, vitamins and pigments compounds) usually plays a crucial role in the oil stability (El-Naggar, 2016).

Tocopherol compounds (includes alpha, beta, delta and gamma tocopherol) play an important role in public health (Chukwuma *et al.,* 2010). Tocopherol content of tiger nut oil compared with that of corn oil is tabulated in the same table, it could be concluded that tiger nut oil contained much higher contents of the determined tocopherols than those for the corn oil, especially delta tocopherol (50mg/ 100g) and gamma (31.3mg/ 100g) tocopherol. Thus, it would be expected to contribute to excellent antioxidant substance. Similar results have been previously reported by Adejuyitan, (2011); and El-Naggar, (2016).

The un-saponifiable matter was analyzed and the percentage of sterols and hydrocarbons are given in Table (2).

Table 1. Unsapnifiable matters and tocopherol compounds of tiger nut and corn oils:

Components / Oils	Unsap. Matter %	Total tocopherol mg/ 100g	Alpha tocopherol mg/ 100g	Beta tocopherol mg/ 100g	Delta tocopherol mg/ 100g	Gamma tocopherol mg/ 100g
Tiger nut oil	0.70	97.4	16.1	-	50	31.3
Corn oil	**0.50**	**14**	**14**	**-**	**-**	**-**

Un-saponifiable matter of tiger nut oil composed of aliphatic hydrocarbon compounds which consisted of C_{25} (38.08%) followed by C_{23} (10.10%) and others aliphatic hydrocarbons were found i.e. C_{14}, $C_{20} – C_{32}$ and squalene. Also, sterol compounds were act beta sitosterol (11.63%), stigma sterol (3.37%), comp sterol (3.15%) and small amount of cholesterol (0.057%) of un-sapnifiable matter. It can be concluded that the tiger nut oil had high content of sterol (21.42%) more than that of corn oil (17.06%).

Table 2. Unsaponifiable matter composition of tiger nut and corn oils.

Hydrocarbons			Sterols		
Component	**Tiger nut oil**	**Corn oil**	**Component**	**Tiger oil**	**Corn oil**
C14	0.70	19.5	Cholesterol	0.0574	0.00
C15	0.00	16.68	Brasicasterol	0.0361	1.690
C16	0.00	29.80	24-Metilen Chol.	0.0127	0.00
C17	0.00	7.80	Comp sterol	3.155	4.233
C18	0.00	0.00	Comp sterol	0.0574	0.00
C19	0.00	0.00	Stigma sterol	3.378	0.00
C20	0.656	0.00	D7-Compesterol	0.178	0.00
C21	0.0	0.00	D7-Stigmasterol	1.236	0.846
C22	2.91	0.00	D7-Avenasterol	0.542	0.00
C23	10.10	0.00	D5-Avenasterol	0.840	0.338
C24	0.86	0.00	Clerosterol	0.174	0.00
C25	38.08	0.00	Beta sitosterol	11.63	9.82
C26	7.37	0.00	Sitostanol	0.1319	0.14
C27	2.319	1.85	Total sterols:	21.42	17.06
C28	3.49	5.85	** Total sterol of un-saponifiable matter		
C29	3.33	0.00			
C30	1.34	0.00			
Squalene	2.78	0.00			
C31	2.35	0.00			
C32	1.51	1.46			
Total hydrocarbons	77.795	82.94			

% Total hydrocarbons of un-saponifiable matter.

Meanwhile, the aliphatic hydrocarbons substance of tiger nut oil was lower (77.795%) than those of corn oil (82.94%). Such results were confirmed with these of El-Naggar, (2016), who showed that total amounts of aliphatic hydrocarbons were found to be slightly higher in the maize oil (82.90). Meanwhile, the dominant compound of tiger nut oil was C25 (38.08%) of total un-saponifiable matter, olive oil was C25 (36.25%), maize oil was C16 (27.30%), sunflower oil was C29 (44.83%) and soy bean was C28 (27.26%).

Fatty acid composition of tiger nut and corn oils:

Fatty acids composition of tiger nut oil compared with that of corn oil was fractionated and determined by GC-MS apparatus. The obtained results are shown in Table (3). Tiger nut oil had a high amount of monounsaturated fatty acid.

Table 3. Fatty acid composition of tiger nut and corn oils.

Fatty acids / Oils	0 :12	0 :14	0 :16	0 :18	0 :20	1 :18	2 :18	3 :18	Sat. fatty acids	Mono unsat. fatty acid	Poly unsat. fatty acid	Total unsat. fatty acids
Tiger nut oil	0.00	1.7	15.4	5.3	6.1	65.8	5.5	0.2	28.5	65.8	5.7	71.5
Corn oil	0.18	10.23	2.14	1.06	0.2	30	50.0	6.10	13.63	30	56.37	86.37

It is worthy mention that the major unsaturated fatty acid was oleic acid (65.8%) followed by palmitic (15.40%) and at the last was linolenic acid (0.2%), meanwhile, saturated fatty acids in tiger nut oil was 28.5%. Thereafter, it is recently gaining greater importance because of its superior stability and healthy safe nutritional benefits. These data agree with those obtained by Imam *et al*., (2013) and Gambo and Da'u, (2014). El-Naggar (2016), found that tiger nut oil has four main fatty acids: linoleic (5.5%), palmitic (15.4%), arachidic (6.1%) and oleic acid being the most abundant (65.8%). Hence, these results indicate that tiger nut oil can be considered as edible oil and essential fatty acids.

Biological effects of tiger nut oil on healthy and hypercholesterolemia rat groups:

It has been known that lipid peroxidation gives complex products including hydroperoxides, cleavage products such as aldehydes exert cytotoxic and genotoxic effects (Simopoules, 2004; Chukwuma *et al.,* 2010).

Lipid peroxidation products have been found in human atherosclerotic lesions, although their pathological significance such as (atherosclerosis and cardiovascular disease including coronary heart disease (CHD)) (Niki *et al*, 2005; Gambo and Da'u, 2014).

The results presented in Table (4), summarized the body weight gain, food intake, food efficiency and relative weight of organs for healthy and treated rat groups, which fed, tested diets at different levels (5, 10 and 15%) of tiger nut oil for 28 days.

Table 4. Biological effects of tiger nut oil on feeding and growth parameters by healthy and cholesterol emic rat .

Treatment	Body weight gain		Food intake per day		Growth rate		Food efficiency		Relative weight of organs (gm%)		
	Zero time	After 28 days	Zero time	After 28 days	Zero time	After 28 days	Zero time	After 28 days	Liver	Heart	Kidney
T_1 (– ve)	120.5	126.96	9.35	11.61	3.55	4.53	0.38	0.39	2.85	0.19	0.55
T_2 (+ve)	126.7	101.8	8.85	10.55	2.50	3.25	0.28	0.30	3.20	0.28	0.65
T_3	118.3	120.5	9.20	11.15	3.50	3.68	0.38	0.33	2.76	0.18	0.54
T_4	126.7	105.45	9.30	10.85	2.65	3.30	0.285	0.304	3.10	0.25	0.60
T_5	119.4	122.75	9.30	11.44	3.25	3.78	0.35	0.330	2.82	0.19	0.54
T_6	126.7	110.4	9.30	10.95	2.80	3.74	0.30	0.341	2.90	0.19	0.66
T_7	120.3	125.22	9.4	11.55	3.4	4.15	0.36	0.36	2.91	0.20	0.56
T_8	126.7	110.0	9.30	10.85	2.90	3.70	0.31	0.341	2.85	0.21	0.67
$LSD_{0.05}$	3.34	3.5	0.45	1.35	0.60	0.4	0.12	0.10	0.18	0.04	0.20

Means values of three replicated. Means in the same column were calculated by different significance (P≤ 0.05) of all treatments. T1= Negative control, T2= Positive control, T3, T5, and T7= Healthy group (5, 10, and 15% of tiger nut oil respectively.), T4,T6,T8= Treated group (5, 10, and 15% of tiger nut oil respectively).

From Table (4), a significant decrease was observed in body weight gain, food intake, growth rate and food efficiency in hypercholesterolemia rat group (positive control) (+ve) as compared with negative control group (-ve). The reduction noticed in body weight gain, food efficiency, food intake and growth rate for rats fed hypercholesterolemia diet may be due to the decreased palatability of dietary mixture (El-Sayed *et al.,* 2004), where pure cholesterol and animal fat were added and increased the energy density of the diet leading to decrease food consumption (Nwodo *et al*., 2014). On the other hand, rats fed on cholesterol enriched diet and supplemented with 5, 10 and 15% levels of tiger nut oil showed a relatively increase in body weight gain, food intake, food efficiency and growth rate compared with healthy rat groups, fed basal diet and supplemented with 5, 10 and 15% of tiger nut oil. Consequently, supplementing diets with tiger nut oil greatly enhanced food intake, food efficiency, growth rate and body weight gain at all levels of supplementation compared with positive control group. Furthermore, it has been recognized that taste and palatability of the diet affected food intake. The results indicate that by increasing tiger nut oil proportion in hypercholesterolemia rat diet the body weight gain, food intake and growth rate increasing. Body weight gain was lower for tiger nut oil as compared with corn oil; this might be due to variation in the constitutional fatty acid (Table 3). The increasing of body weight gain, food intake, food efficiency and growth rate in treated rat groups may be due to the higher content of phytochemical compounds and monounsaturated fatty acids occurred in tiger nut oil than more of corn oil, and its effect on protein metabolism by activation some enzymatic pathway, the activity effect caused increasing in protein absorption and consequently an increase in weight gain, food intake, food efficiency and growth rate (Li *et al.,* 2011). Generally, as a result of the lower food intake, growth rate of the hypercholesterolemia rats group and higher body weight gain of the healthy rat groups and negative control group, values for food efficiency were higher than those of hypercholesterolemia rats group. The obtained results are in agreement with those reported by Ghasi *et al., (*2000); Bamishaiye *et al., (*2010); Anthony and Ashawe, (2014); Oseni *et al., (*2015); Atef and Al-Rethea, (2016); and Mohamed, (2016). This is also in accordance with Alam *et al.,* (2011), who found that feeding a diet containing 5% powder of mushroom to hypercholesterolemia rats significantly reduced weight gain in hypercholesterolemia rats.

Concerning the change in internal organ weight expressed as a percentage of body weight (organ/ body ratio, gm/100 gm), during feeding experiment for 28 days using tested diets. Results in the same Table showed that small and variable difference was observed in relative weight of organs when used tested diets.

Regarding to the relative weight of organs, it appears a significant difference ($P \leq 0.05$) between positive control (+ve) group and treated rats fed hypercholesterolemia diet and supplemented with 5, 10 and 15% levels of tiger nut oil. While, there are non-significant differences ($P \leq 0.05$) between negative control group and treated rats fed hypercholesterolemia diet and supplemented either with 10% or 15% of tiger nut oil. Obtained results agreed with those of Mohamed, (2016) and Adewuma and Samson, (2016) who reported that the relative weight of organs for treated rats fed either *Moringa oleifera* seed oil or their leave extract decreased during 4 weeks. Due to the higher phenolic and antioxidants content of *M. oleifera* seed oil and their leave extract.

Influence of tiger nut oil on liver and kidney functions:

Results in Table (5) illustrate a significant increase ($P \leq 0.05$) for both of serum glutamic pyruvic transaminase (GPT) and serum glutamic oxaloacetic.

Table 5. Influence of tiger nut oil on liver and kidney functions of the healthy and hypercholesterolemic rats.

Treatments	GOT IU/L		GPT IU/L		Creatinine mg/dl		Blood urea mg/dl		Uric acid mg/dl	
	Zero time	After 28 days	Zero time	After 28 days	Zero time	After 28 days	Zero time	After 28 days	Zero time	After 28 days
T_1 (– ve)	38.5	40.5	32.0	34.0	0.75	0.80	19.50	20.50	3.00	2.95
T_2 (+ve)	75.5	66.4	55.8	52.7	1.45	1.40	33.50	30.20	6.35	6.20
T_3	39.3	40.0	32.0	33.0	0.75	0.75	20.0	21.30	3.05	3.10
T_4	74.8	60.2	55.5	48.3	1.44	1.30	33.7	25.15	6.35	5.10
T_5	38.0	38.5	31.5	30.0	0.74	0.72	19.50	20.0	3.10	2.80
T_6	75.0	48.0	54.7	43.0	1.45	1.20	33.50	22.0	6.30	4.75
T_7	38.3	43.0	32.3	35.0	0.75	0.85	20.10	20.0	3.00	3.90
T_8	74.5	55.0	56.1	43.0	1.45	1.10	33.50	21.0	6.40	4.20
L.S.D	4.47	5.11	3.55	3.21	0.65	0.55	3.12	2.02	1.65	1.84

Means values of three replicated. Means in the same column were calculated by different significance (P≤ 0.05) of all treatments. T1=Negative control T2= Positive control T3, T5, and T7=Healthy groups (5, 10, and 15%of tiger nut oil respectively). T4, T6, and T8=Treated groups (5, 10, and 15% of tiger nut oil respectively).

Transaminase (GOT) enzyme activities in hypercholesterolemia rats group (positive control) as compared with healthy rat groups or negative control group (–ve). These data indicates that the serum activities of GOT and GPT increased from 38.5 and 32 **IU/L** in positive rats group by a percentage of 96.10 and 74.37% respectively. The current observation may reflect partial liver cell damage, which induced by hypercholesterolemia diet. The liver functional enzymes GOT and GPT activities in experimental animal bloods are considered the excellent marker of liver dysfunctions and damages which caused by exposure to the toxic substances (Abd-El-Ghany, 2006; EL-Naggar, 2007). Results showed that after 28 days, the serum GOT and GPT activities in treated rat groups were gradually decreased by increasing the concentration of tiger nut oil in the diet, compared with hypercholesterolemia rats group (+ve). The results indicate that the serum GOT and GPT activities relatively decreased from 75 and 54.7 **IU/L** in treated rats group with 10% of tiger nut oil (T_6) by a percentage of 36 and 21.4% respectively. Herein, data indicates that as tiger nut oil level in hypercholesterolemia rat diet increased, the GOT and GPT activities are decreased. Thereupon, tiger nut oil may be enhancing the detoxicated properties of the liver. The enhancement in liver function enzymes activities with addition of fresh oil to the diet, tiger nut oil could be attributed to their higher content of monounsaturated fatty acid and phytochemicals. These benefits substances are providing the protection against the lipid oxidation in liver (Badawy and Hegazi, 2004; Gambo and Da' u, 2014; Mohamed, 2016).

Consequently, tiger nut oil and corn oil diets were improved liver functional enzymes activity. These results are in agreement with those of Mohamed and El-Metwally, (2004), who proved that treatment with Vit. E significantly inhibited lipid peroxidation as well as liver cell damage. The decrease in serum GOT and GPT activities for treated rat groups may be attributed to the decrease in oxidative stress through the antioxidants properties of Vit. E. Meanwhile, the increase in serum activities of GOT and GPT for hypercholesterolemia rats group may be attributed to the toxic liver damage induced by cholesterol emic diet (Mohamed, 2016).

In this respect, Mohamed, (2016) found that feeding a diet containing 5, 10 and 15% of *M. oleifera* seed oil or 200, 400 and 600 mg of its leave extract to hypercholesteremic rat had statistical significant ($P \leq 0.05$) difference on GPT and GOT activities.

With respect to kidney functions, it can be observed from the same table that creatinine, blood urea and uric acid levels are elevated in hypercholesterolemia rats group. This elevation of serum creatinine in hypercholesterolemia rats group may be due to the accumulation of deleterious toxic compounds possible formed by lipid peroxidation during the feeding on hyperlipidemic diets (Niki *et al.,* 2005; Bamishaiye *et al.,* 2010).

It is worthy mention that the treated rat groups fed cholesterol enriched diet and supplemented with different levels of tiger nut oil cleared reduce in creatinine, blood urea and uric acid compared with hypercholesterolemia rats group. The lowest creatinine (1.10mg/ dl), blood urea (21mg/ dl) and uric acid (4.20 mg/ dl) were recorded for rats fed on hypercholesterolemia diet and supplemented with 15% of tiger nut oil (T_8). The results indicate that creatinine, blood urea and uric acid relatively decreased from 1.45, 33.5 and 6.4mg/dl in the treated rats group with 15% of tiger oil (T_8) to 1.10, 21 and 4.20 mg/ dl by a percentage of 24.14, 37.31 and 34.4% respectively. Thereupon, increasing the level of tiger nut oil addition showed a lower value of creatinine, blood urea and uric acid. These results are in the same line with those of Mohamed, (2016), who found that when treated rats fed diet containing 5, 10 and 15% *M. oleifera* oil or 200, 400 and 600 mg of its leave extract for 4 weeks, the kidney functions were improved.

In this respect, Alam *et al.,* (2011) found that feeding hypercholesterolemia rats a diet containing 5% powder of *pleurotus ostreatus* fruiting bodies had no effects on creatinine, blood urea and uric acid.

Influence of tiger nut oil on lipid profile:

Hyperlipidemia, a major modifiable risk factor for atherosclerosis and cardiovascular disease, including CHD. Very high levels of lipids or TG can cause yellowish nodules of fat in the skin beneath eyes, pain, swelling of liver, spleen or pancreas or whitish rings around the eye's iris occur (Niki *et al.,* 2005; Mohamed, 2016).

The results in Table (6) show a drastically increase in total lipids, total cholesterol, triglycerides TG, low and very low-density lipoprotein cholesterol (LDL and vLDL – cholesterol) in hypercholesterolemia rats group, compared with negative control or healthy rat groups. On the other hand, results show a reduction in increase rate of high density lipoprotein cholesterol (HDL-cholesterol) in hypercholesterolemia rats group. These data indicate that the serum total lipids, cholesterol, TG, LDL and vLDL-cholesterol increased from 340, 110, 95.3, 70.5 and 18.8mg/dl in negative rats group (–ve) to 450, 150, 170.5, 128 and 28mg/dl in positive rats group (+ve) by a percentage 32.35, 36.36, 78.5, 82.27 and 48.9% respectively. This elevation attributed to lipid hydroperoxides, which formed as the major primary products, however they are substrate for various enzymes and they also undergo various secondary reactions (Sanchez-Muniz *et al.,* 1991; Usman and Hosono, 2000; Abd-EL-Fattah, 2007; Chukwuma *et al.,* 2010; Hasan *et al.,* 2013).

Regarding to the treated rat groups fed positive diet and supplemented with different levels of tiger nut oil illustrated reduce in total lipids (365mg/ dl) for T_8 group, total cholesterol (140mg/ dl) for T_6 group, TG (130.45mg/ dl) for T_6 group, LDL-cholesterol (80.31mg/ dl) for T_6 group and vLDL-cholesterol (26.09mg/ dl) for T_6 group, nevertheless increase in HDL-cholesterol (36.41mg/ dl) for T_4 group.

Consequently, after 28 days the serum total lipids, cholesterol, TG, LDL-and vLDL-cholesterol levels in hypercholesterolemia rat groups treated with 5, 10 and 15% of tiger nut oil were significantly decreased by increasing the level of tiger nut oil in the diet. It means that basal diet containing tiger nut oil has more efficiency for decreasing the serum lipids, cholesterol, TG, LDL and vLDL-

cholesterol than basal diet does not contain tiger nut oil.

Table (6): Influence of tiger nut oil on lipid profiles of the healthy and hypercholesterolemia rats.

Treatment	Total lipid mg/ dl		Total choles-terol mg/ dl		Triglyceride mg/ dl		HDL-chol. mg/ dl		.LDL-chol		.vLDL-chol		A therogenic index	
	Zero time	After 28 days	Zero time	After 28 days	Zero time	After 28 days	Zero time	After 28 days	Zero time	After 28 days	Zero time	After 28 days	Zero time	After 28 days
T_1	340	350	110	120.5	95.3	99.15	27.5	28.5	70.5	72.17	18.8	19.83	3.24	3.22
T_2	450	440	150	188.10	170.5	145.4	29.5	33.60	128	125.42	28	29.08	5.28	4.59
T_3	344	360	112	125.7	93	105.23	27	27.65	71.8	76.95	20.4	21.10	3.41	3.54
T_4	451	420	144	165.5	170.4	135.35	29.4	36.41	115	102.02	25	27.07	4.76	3.54
T_5	340	340	108	110.25	92	90.10	26.5	27.56	70.5	64.67	17.5	18.92	3.32	3.03
T_6	450	370	148	140	171.3	130.45	30	33.6	108	80.31	25.3	26.09	4.44	3.16
T_7	345	365	107	130.8	95.5	110.40	27	27.46	72.1	81.26	19.3	22.08	3.38	3.76
T_8	455	365	152	150	170.2	130.5	30	30	105	93.9	25.4	26.1	4.34	4
$LSD_{0.05}$	28	22	18.4	20	21.5	11.45	1.75	3.25	30.7	29.5	6.5	7.3		

Means values of three replicated. Means in the same column were calculated by different significance ($P \leq 0.05$) of all treatments.

T_1= Negative control T_2= Positive control T_3, T_5, and T_7=Healthy groups (5, 10, and 15%of tiger nut oil respectively). T_4, T_6, and T_8=Treated groups (5, 10, and 15%of tiger nut oil respectively).

Results should that lipid profile has appeared significant differences ($P \leq 0.05$) between positive control group (+ve) and treated rats fed hypercholesterolemia diet and supplemented with different levels of tiger nut oil. Meanwhile, there were small and variable differences observed between negative control group and treated rats fed hypercholesterolemia diet and supplemented with 10% or 15% of tiger nut oil. Obtained results were nearly similar with those obtained by Mishral and Singh, (2010); Handayani, *et al., (*2011); Priya and Chellaram, (2011) and El-Bushuty and Shanshan, (2012;), who found that methanolic extract of mushroom reduced the total lipids, total cholesterol, T.G, LDL and vLDL-cholesterol in hyperlipidemic group of albino rats.

In this respect, Mohamed, (2016) found that feeding a diet containing 5, 10 and 15% of *M. oleifera* seed oil and/ or 200, 400 and 600 mg of its leave extract reduced the lipid profiles in hypercholesterolemia rats group without any deleterious effect on liver and kidney. Because lipid peroxidation induced by singlet oxygen inhibited by the inhibition of its formation by quenching of the singlet oxygen itself by antioxidants (Chukwuma *et al.,* 2010). Tiger nut oil contains various radical-scavenging antioxidants (Hasan *et al.,* 2013). Antioxidant defenses may be through prevention of the formation of active oxidants (Ismail and Saad, 2004), scavenging, quenching and removal of active oxidants (Chukwuma *et al.,* 2010), repair of damage and excretion of toxic oxidation products, and adaptive responses (El-Sayed, *et al.,* 2000; Usman and Hosono, 2000; Niki *et al*., 2005) on the other word, the inhibition of enzymatic lipid oxidation may be achieved by inhibition of either the activation or reaction of an enzyme (Mohamed, 2016). Vitamin E, scavenges lipid peroxyl radicals, chain carrying species in the lipid peroxidation, to break chain propagation (Niki *et al.,* 2005).

It is worth mentioning that the healthy rats group, fed on basal diet supplemented with 10% of tiger nut oil showed a significant decrease in the reduction rate of LDL-cholesterol, and slight increase in

the increasing rate of HDL-cholesterol as compared with 5% or 15% of tiger nut oil levels.

Also, from the same table, data showed a great increasing in atherogenic index (AI) in hypercholesterolemia rats group compared with negative control group. Healthy rat groups fed basal diet and supplemented with different levels of tiger nut oil showed a decrease in AI as compared with negative control group (–ve) or positive control group (+ve). Consequently, treated rats fed on hypercholesterolemia diet and supplemented with 10% level of tiger nut oil showed legible decreasing in total lipid (370mg/dl), total cholesterol (140mg/dl), TG (130.45mg/dl), LDL-c (80.31mg/ dl), vLDL-C (26.09 mg/ dl) and AI (3.16) than that observed for treated rats fed on hypercholesterolemia diet and supplemented either with 5% or 15% of tiger nut oil. This study concluded that tiger nut oil meal-based diet in rats decrease lipid profiles and development of aortic atherosclerosis when compared with corn oil meal-based diet. Thereafter, hypercholesterolemia increased the risk of increased LDL-c + vLDL-c/ HDL-c[(AI). The development of CHD is highly correlated with elevated serum cholesterol, in particular in LDL-fraction which is the major vehicle cholesterol transport in human blood (El-Sayed *et al.,* 2000; EL-Naggar, 2007; Oseni *et al.,* 2015).

The results agree with those of Mohamed, (2016) who investigated the effect of *M. oleifera* oil and/ or its leave extract on maintaining a reduction of AI. That the *M. oleifera* oil and its leave extract groups showed a significant reduction in LDL-cholesterol but, more important a pronounced in HDL-cholesterol. The use of vegetable oils which containing unstifled phytosterols for cholesterol – lowering purposes was done by many researchers (EL-Newary *et al.,* 2011; Mohamed, 2016), they showed that the mechanism of phytosterol actions is based on its ability to reduce cholesterol absorption, owing to co precipitation of cholesterol and phytosterols and to competition for space in mixed micelles. Herein, tiger nut oil contains higher total sterols (21.42% of un-saponifiable matter) than corn oil (17.0%).

Conclusion:

The present work shows that *Cyperus esculentus* L. oil has chemical and fatty acids composition make it ideally suited as a nutritional defense against lipid oxidation. As showed above *C. esculentus* oil is high in monounsaturated fatty acid (65.8%) and total tocopherol (97.4mg/ 100gm of oil), especially delta (50mg/ 100gm) gamma (31.3mg/ 100gm) and alpha (16.1mg/ 100gm), and carries a lower risk of oxidation than corn oil, since as above mentioned that tiger nut oil is low in polyunsaturated fatty acid (5.7%) compared with corn oil (56.37%). Thereupon, *Cyperus esculentus* L. oil can be used to be more effective than corn oil as hypocholesterolemia agent.

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التأثير البيولوجي لتناول زيت حب العزيز (*Cyperus esculentus* L.) على الفئران الأصحاء والفئران المصابة بارتفاع الكوليستيرول

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الملخص

هدف البحث إلى دراسة تأثير زيت حب العزيز في الفئران الأصحّاء والفئران المصابة بارتفاع الكوليسترول وتأثير ذلك في الوزن المكتسب والغذاء المتناول وكفاءة التحويل والوزن النسبي للأعضاء الداخلية والتحليلات البيولوجيّة. أجريت الدراسة على 48 فأر من ذكور فئران الألبينو بوزن (150±5 غ)، وقسّمت إلى ثمانِ مجموعات، أربع مجموعات تضمّ الفئران الأصحاء (T1، وT3، و T5 وT7) واختيرت منها مجموعة واحدة (T_1) كمجموعة شاهد غير مصابة قياسيّة سالبة، حيث تمّ تغذيتها على الغذاء الأساسي فقط، بينما تغذت الثلاث مجموعات المتبقية على الغذاء الأساسي مدعّماً بثلاثة مستويات مختلفة من زيت حب العزيز (15، و10، و15 %) لمدة أربعة أسابيع. وأمّا الأربع مجموعات الأخرى المصابة بارتفاع الكوليستيرول (T_2، وT_4، وT_6، وT_8) اختيرت منها مجموعة واحدة (T_2) كمجموعة شاهد مصابة بارتفاع الكوليسترول قياسيّة موجبة، حيث تمّ تغذيتها على غذاء أساسي غني بالكوليستيرول (1 % كوليسترول+ 0.5 % حمض كوليك)، بينما غُذّيت الثلاث مجموعات المتبقيّة على الغذاء الغني بالكوليستيرول والمدعّم بثلاثة مستويات مختلفة من زيت حب العزيز (5، و10 و15 %) لمدة أربعة أسابيع. وأوضحت النتائج أنّ المجموعات المصابة بارتفاع الكوليستيرول والمدعّمة بالمستويات المختلفة من زيت حب العزيز تباينت في زيادة الوزن المكتسب، والغذاء المتناول، ومعدّل النمو. كما أوضحت النتائج وجود فروق معنويّة بين مجموعة الفئران المصابة والمجموعات المعالجة بمستويات مختلفة من زيت حب العزيز. كما أبدت النتائج عدم وجود فروق معنويّة بين المجموعة الشاهد غير المصابة القياسيّة السّالبة والمجموعات الأصحاء. وأظهرت النتائج وجود نقص معنوي في قيم أنزيمات الكبد، والكرياتينين، ويوريا الدم، وحمض اليوريك للمجموعات المعاملة، مقارنةً بالمجموعات الأصحاء أو المجموعة الشاهد المصابة بارتفاع الكوليستيرول. وبينت النتائج أن المجموعة المصابة والمعاملة بمستويات مختلفة من زيت حب العزيز قد أبدت نقصًا معنويًا في قيم ليبيدات سيرم الدم، والكوليستيرول الكلي، والجليسريدات الثلاثية، والكوليستيرول منخفض الكثافة، ومعامل الخطورة. بينما أبدت النتائج ارتفاعًا ملحوظاً في الكوليسترول مرتفع الكثافة. كما أوضحت النتائج أنّ التركيب الكيميائي لزيت حب العزيز من الأحماض الدهنيّة يجعله غذاءً مناسبًا لمقاومة الأكسدة الداخليّة المسئولة عن تصنيع الكوليسترول بالكبد. ومن هنا توصي الدّراسة باستخدام الوجبات المدعمة بزيت حب العزيز للتغلب على مشكلات ارتفاع الكوليستيرول، إلى جانب تحسين وظائف كلًا من الكبد والكلى.

الكلمات المفتاحية: الخصائص الحيوية، التركيب الكيميائي، زيت حب العزيز، ارتفاع الكوليسترول.